

Serial No. 10/091,912

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In the Specification:

Please replace the Title as follows:

~~High Throughput Mutagenesis Screening Method~~

Mutant *Pseudomonas mendocina* Cutinase

On page 7, please amend the paragraph at lines 7-14 as follows:

Following selection of the subset of amino acid positions in the protein for mutagenesis (step 2 in Fig. 2), site-saturation libraries are made at the selected sites by random substitutions of all 19 amino acids using conventional oligonucleotide-directed mutagenesis (step 4 in Fig. 1). Such conventional library formation is well known to those skilled in the art and was carried out generally as described by Airaksinen, A and Hovi, T. (1998) Nucleic Acids Research, Vol. 26, No. 2: 576-581. Other site-saturation methods may be used and kits, such as the Stratagene Quik-Change™ QUIK-CHANGE® kit or the Sculptor Mutagenesis Kit (Amersham, UK) will be apparent to those skilled in the art.

On page 10, please amend the paragraph at lines 14-25 as follows:

Site-saturation variant libraries were created at amino acid positions corresponding to residue positions 57-66, 68, 85, 86, 88, 125-127, 130, 148-152, 154, 155, 176-183, and 204-211 of SEQ ID NO:2 in *P. mendocina* cutinase, expressed in *Bacillus subtilis* strain 3934. The sequence of the parent cutinase is attached hereto as Fig. 17. Libraries were created with the Stratagene Quik-Change™ QUIK-CHANGE® kit using oligonucleotide primers randomized with NN(G/C) at the target position. Each selected amino acid was randomly replaced with all 19 possible alternatives. Colonies obtained from the variant libraries were selected and placed into 96-well growth plates that contained 100 µL/well of MOPS 1A starter medium. The plates were incubated at 37°C overnight with continuous humidified shaking at 260 rpm. A sample from each well was replicated into a Millipore 96-well filter plate which contained 200 µL/well of MOPS-Urea medium. 50 µL of 45% glycerol was added to each well of the initial growth plates, which then were stored at -70°C.

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On page 13, please amend the paragraph at lines 20-32 as follows:

The results from the TCA assay, the PET hydrolysis assay, and the stability assay were calculated, using conventional, known formulas as set out above, using Excel[™] EXCEL® spreadsheet software. Variants were graded and ranked for stability and hydrolysis activity. Table 1 shows the results obtained from the site-saturation mutagenesis procedure described in this example. The results were graded and color coded as follows: green (■) = more than 2% of the variants at the listed sites had significantly greater activity than the control (beneficial mutation); blue (■) = the majority of the variants had unchanged activity (neutral mutation); red (■) = the majority of the variants had significantly decreased activity relative to the control (detrimental mutation); and brown (■) = approximately half of the variants had unchanged activity and half had decreased activity (neutral mutation). The color coding was repeated using the same color shading and same percentage criteria for stability results. As can be seen in Table 1, color coding easily identifies those variants having both desired enzyme properties.

On pages 13-14, please amend the legend to Table 1 as follows:

Table 1 shows the results obtained from a typical enzyme system. The results are color-coded as follows: Green (■) – more than 2% of the clones had significantly greater activity than control. Blue (■) – the majority were unchanged. Red (■) – the majority had significantly decreased activity relative to the control. Brown (■) – approximately half were unchanged and half were decreased.